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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/643,755	08/23/2000	Gijs van Rooijen	9369-153/MG	1008

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EXAMINER	
HELMER, GEORGIA L	
ART UNIT	**PAPER NUMBER**
1638	

DATE MAILED: 09/27/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/643,755	VAN ROOIJEN ET AL.	
	Examiner	Art Unit	
	Georgia Helmer	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 June 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3,5-17 and 21-23* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,5-17 and 21-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

REQUEST FOR CONTINUED EXAMINATION

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 29 June 2006 has been entered.

Status of the Claims

2. Applicant has amended claims 1 and 17. Claims 1, 3, 5-17, and 21 -23 are pending, and are examined in the instant action.

3. All rejections not addressed below have been withdrawn.

4. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claim Rejections - 35 USC § 103

5. Claims 1, 3, 5-17 and 21-23 are rejected under 35 U.S.C. 103 as being unpatentable over Willmitzer et al (WO 92/01042) in view of Kusnadi, et al., 1998, (Biotech. Bioeng, Vol 60, No. 1, pages 44-52) and Applicant's admitted prior art.

Willmitzer teaches a method for the production of chymosin in a plant seed comprising introducing into tobacco and potato plant cells a chimeric

nucleic acid sequence comprising a seed-specific phaseolin promoter, a nucleic acid sequence encoding pro-peptide chymosin, and a terminator, then growing the plant until it sets seeds and obtaining chymosin-containing seeds (Abstract, p 4, 5, 10 and 13). Seeds obtained from the transgenic plants are tested to assure that the gene of interest is present. The expressed enzyme can be isolated from the seed (p 3). Willmitzer further teaches including a plant signal sequence (p 5). The pro-chymosin of Willmitzer appears to a mammalian chymosin obtainable from a bovine, sheep, or goat source (p.13), since these are the only know nature sources of chymosin (specification, p.1). Willmitzer teaches the production of 0.1% - 0.5% chymosin of the total soluble protein (p. 14, lines 30-32). Since the method of Willmitzer is the same as Applicant's method, and teach the same promoter as preferred by Applicant, the percentage yields would have been an inherent property of the DNA construct used. If Applicant's percentage yields are different from that of Willmitzer, it is suggested that Applicant amend the claims and include specific structures such which would account for this difference.

Willmitzer further teaches a method of isolating chymosin by crushing (p 12, line 10) plant tissue, fractionating the resulting product (p 12, lines 9-15), contacting this product with a protein binding resin (p 12, lines 20-25).

While Willmitzer teaches the inclusion of a plant signal sequence and terminator in a chimeric construct, Willmitzer does not specifically teach a tobacco PR-S signal sequence and phaseolin terminator. However, the inclusion of a heterologous signal sequence and terminator in a chimeric construct was

notoriously well know in the art, as evidenced by the numerous examples set for by Willmitzer (p. 5) as well as by Applicant (p. 9 and 12). Applicant's admitted prior art indicates that a tobacco PR-S signal sequence and phaseolin terminator, as well as their biological properties, were also known at the time the invention was made (p. 9 and 12).

Willmitzer et al does not teach production of chymosin from transgenic seed by fractionating crushed transgenic plant seed into an oil fraction, an aqueous fraction and an insoluble fraction.

Kusnadi, et al. (Abs, p. 50, 2nd column final full ¶, and p. 51) teach production of beta-glucuronidase (rGUS) enzyme in transgenic corn seed by fractionating crushed plant seed into an oil fraction, an aqueous fraction and an insoluble fraction. The motivation to combine Kusnadi, et al. with Willmitzer et al is provided by Kusnadi, et al. "Alternatively, corn oil could be extracted with hexane from the initial material or removed by a diafiltration from the crude extract." "The analysis of rGUS purification indicates that except for the oil in the germ extract... the crude extracts would be purified similarly to clarified cell extract from microbial fermentation. In other words, after removing the oil and/or starch from the extracts, the know-how from purifying microbial protein products could be directly applied to the purification of recombinant proteins from the transgenic corn." See p. 52.

Accordingly, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to utilize any of the known plant signal sequences and terminators of the prior art, including the claimed tobacco

PR-S signal sequence and phaseolin terminator, for their known biological properties, in the chimeric construct for expressing the chymosin of Willmitzer without any surprising or unexpected results. One skilled in the art would have been motivated to generate the claimed invention with a reasonable expectation of success.

Furthermore, it would have been obvious to one of ordinary skill in the art, at the time of the invention was made, to substitute the transgenic corn plant seeds of Kusnadi, et al. for the transgenic plant leaves of Willmitzer et al, to produce and isolate chymosin from transgenic seeds comprising an oil fraction, without any surprising or unexpected results.

Applicant traverses that "Willmitzer et al do not produce or isolate chymosin from plant seeds and do not teach how to isolate chymosin from plant seeds...there is nothing in Willmitzer et al that would motivate one of skill in the art to develop and isolate method as described in the claims". (Response, p. 8, 1st ¶). Applicant argues that of the three criteria for prima facie case of obviousness: suggestion or motivation to modify, reasonable expectation of success, and teaching or suggesting all the claim limitations, "none...are met by Willmitzer et al or Applicant's admitted prior art." (Response of 29 June 2006, p. 7) Applicant continues: "With respect to motivation, the Examiner refers to the Abstract and p. 9, lines 32-p.10, line 8 of Willmitzer et al. The Abstract of Willmitzer et al does indicate that the enzyme may be recovered from the plant. Applicant traverses that the PAGE (polyacrylamide gel electrophoresis) used by

Willmitzer et al is not a protein binding resin and is a destructive method, rendering proteins non-functional and not useful for commercial purposes. Applicant continues that "one of skill in the art would know the Willmitzer et al PAGE is detecting an individual protein in a complex mix and not using it to recover and purify chymosin from a total extract. (Response, p. 8, 2nd ¶).

Applicant's traversal is unpersuasive. Willmitzer et al purified chymosin from a total extract of proteins using PAGE which separates proteins based on charge and molecular size, as discussed above. Separating a protein from a mix of proteins is the process of purifying or isolating that protein. Thus chymosin protein was purified/isolated from transgenic tobacco leaf tissue. Willmitzer et al (p. 12, lines 7-25) teach, "total protein extracts were first separated according to size in the presence of SDS. After the separation proteins were transferred on a nitrocellulose membrane and subsequently analyzed for specific proteins.by incubating the nitrocellulose with and antibody specific for the protein...(line 25). Applicant's arguments relative to denaturing conditions and commercial usage are not in accord with the scope of the claims, which are drawn to chymosin. No claim is made to "native" chymosin, or biologically active chymosin, or specific enzymatic activity of the chymosin.

Applicant traverses primarily Willmitzer et al teach detecting chymosin in plant leaves. "One of skill in the art would not expect a method of preparation from leaves to be successful when purifying the protein from seeds with an oil fraction, citing the specification (p. 22, lines 1-16) saying that it is desirable to

remove the oil fraction when chymosin is produced from seed with a relative high oil content". (Response, p. 8 ¶ bridging p. 9)

Applicant's traversal is unpersuasive. There is no reason why one of skill in the art would not expect a protein purification from leaves to work for a purification of the same protein from seeds. On the contrary, it is the particular protein to be purified which largely determines the appropriate purification method. Since proteins have been purified from leaves and seeds and chymosin is a well known and well characterized enzyme (see specification p. 2, lines 1-21), one of skill in the art would not expect a surprising or unexpected result for purifying chymosin. Indeed, the 37 CRF §1.132 Declaration of Dr. David Dennis (dated 26 May 2004) says as much. Dennis says, "The methodologies of protein purification have be developed and established for each protein that is purified". "The purification of a protein from plant tissue to homogeneity is not a simple or routine task. It is something that has to be developed for each protein that is isolated."

Applicant traverses primarily that purification of recombinant proteins from oil seeds is difficult due to the large quantities of oil. "The art-recognized solution was to extract the oil using conventional hexane extraction procedures. However the hexanes and organics denatures proteins. Therefore, one of skill in the art would not expect to have success purifying chymosin from leaves using hexane. (Response, p. 9, ¶ bridging from p. 8).

Applicant's traversal is unpersuasive. These "art-recognized problems" are not presented or alluded to in the specification. Therefore a discussion of these

issues is not in accord with the scope of the claims. The claims are drawn to "chymosin". No claims are drawn to "native" chymosin, or "active" or "biologically active" chymosin, or "enzymatically active" chymosin.

Furthermore, Kusnadi et. al. studying processing of transgenic corn seed and its effect on the recovery of recombinant beta-glucuronidase (rGUS), dry-milled, fractionated and hexane-extracted transgenic corn seed to produce full-fat and defatted germ fractions. They found that the extraction of corn oil from ground germ with hot hexane (60 deg C) did not affect the extractable rGUS activity (Abstract).

Applicant traverses primarily that Willmitzer et al do not recover chymosin from a protein binding resin. Rather Willmitzer et al uses PAGE. (Response, p. 9)

Applicant's traversal is unpersuasive. Willmitzer et al teach extraction of chymosin from transgenic plant tissue using the steps of homogenization, centrifugation to produce an aqueous fraction, and PAGE separation of the proteins. After this separation, the proteins were transferred to a nitrocellulose membrane and subsequently incubated with an antibody specific for the protein to be detected. (Willmitzer et al , p. 12 lines 8-32) Nitrocellulose is a protein binding resin. In fact, *"[t]he ability to bind to nitrocellulose is commonly accepted as being a universal property of proteins and has been widely used in many different fields of study*" [emphasis added]. See Oehler et al, 1999. Therefore, Willmitzer taught recovering chymosin from a protein binding resin.

Accordingly, the claimed invention is prima facie obvious in view of the prior art.

Applicant traverses primarily the state of the art at the time of the invention supports the inventiveness of the claims. At the time of the invention and even for years after the present application was filed in August 2000, it was abundantly clear that the recovery and purification of recombinant proteins from plants is a distinct challenge from the synthesis of recombinant proteins in plants. Applicant cites Borisjuk et. al.1999, saying "The large-scale production of recombinant proteins in plants is limited by relatively low yield and difficulties in extraction and purification." (Response, p. 11)

Applicant's traversal is unpersuasive. Borisjuk et. al. continue "To overcome this problem, secretion-based systems utilizing transgenic plant cell or plan t. organs asceptically cultivated in vitro have been investigated." They propose production of recombinant proteins by rhizosecretion. "The rhizosecretion system offers a simplified and more cost-effective method for isolating recombinant protein from a simple hydroponic medium rather than from complex plant extracts." (p. 468. 2nd column).

Applicant traverses primarily that Komarnytsky, et al, Plant Phys. 2000, states that protein extraction and purification ...is an important obstacle in large-scale produciton in plants.. Applicant's traversal is unpersuasive. Komarnytsky, et. al. continue "to partially overcome this problem , a rhizosecretion system for the production of recombinant protein has been developed. They further propose

the production of recombinant protein in tobacco guttation fluid. "Guttation, the loss of water and dissolved materials from uninjured plant organs, is a common phenonemon in plants. Guttation fluid has the potential of increasing the efficiency of recombinant protein technology by increasing yield, abolishing extraction and simplying its downstream processing." Abstract, p927. Komarnytsky, et. al. continue that "there has been significant success in tackingly some of the limitations of plant bioreactors, such as low yields and inconsistent product quality, that have limited the approval of plant derived pharmaceuticals".

Applicant traverses primarily that Menhaus et. al., 2004, talks about consideraitons for the recovery of recombinant proteins from plants in terms of costs, citing specific language.

Applicant's traversal is unpersuasive. Menhaus et. al. state that "tradiitional processing operations such as flaking and milling work well for the transgenic plant material and do not compromise the integrity of the recombinant protein, regardless of whether it is targeted intracellulaerly or secreted. Furthermore, the oil extraction of *ground canola seed or corn germ* with hexanes has not had a detrimental effect on the one target protein studied." [Emphasis added]. See p. 1007, 2nd column.

Applicant traverses primarily that "[t]herefore, at the time of the invention, there was nothing in the state of the art that would lead one of skill in the art to the present invention." (Response, p. 12)

Applicant's traversal is unpersuasive. Willmitzer et al teach recovery of chymosin from transgenic tobacco tissue. Kusnadi, et al. teach the recovery of rGUS from transgenic corn seed (an oil seed) and provide the motivation to combine Willmitzer et al with Kusnadi, et al.

Applicant traverses, requesting the reconsideration of the 37 CRF §1.132 Declaration of Dr. David Dennis (dated 26 May 2004). (Response, p. 13). Dennis says, "The methodologies of protein purification have be developed and established for each protein that is purified. This is especially true of the purification of chymosin from plant seeds where pure active protein is required in large amounts.... These method steps are not obvious in the light of Willmitzer."

Applicant's traversal is unpersuasive. No claims are drawn to "active" or "pure" chymosin. The scope of the argument is not in accord with the scope of the claims. Additionally, the instant §103 rejection is made over Willmitzer et al in view of Kusnadi, et al.

Also, see Voisnet v. Coglianese and McCorkle, 173 USPQ 16 (CCPA 1972), which teaches that the opinion testimony of an expert witness does not establish any material fact and may be rejected in favor of other evidence.

Remarks

6. No claims are allowed

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Georgia L. Helmer whose telephone number

is 571-272-0796. The examiner can normally be reached on M-Th, 10:30 AM-6:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Marie Grunberg can be reached on 571-272-0975. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Georgia Helmer PhD
Patent Examiner
Art Unit 1638
15 September 2006

ELIZABETH MCELWAIN
PRIMARY EXAMINER

for Phuong Bui